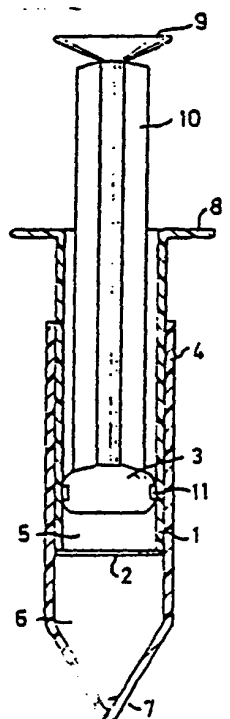


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(21) International Application Number: PCT/SE81/00199 (22) International Filing Date: 30 June 1981 (30.06.81) (31) Priority Application Number: 8005023-0 (32) Priority Date: 8 July 1980 (08.07.80) (33) Priority Country: SE (71) Applicant; and (72) Inventor: STENKVIST, Björn, Gunnar [SE/SE]; Döbeinsgatan 13, S-752 37 Uppsala (SE). (74) Agents: EKELÖF, Carl, Herman et al.; H Albinns Patentbyrå AB, Box 7664, S-103 94 Stockholm (SE).	(81) Designated States: DE, DE (European patent), DK, FI, FR (European patent), GB, GB (European patent), JP, NO, US. Published With international search report With amended claims	
(54) Title: PROCESS AND DEVICE FOR PREPARATION OF CELL SAMPLES FOR CYTOLOGICAL TESTS (57) Abstract <p>Process for preparing cell samples for cytological tests of exfoliated cells, in which the cell sample is in the form of a slurry in a physiological solution, which is introduced into a first chamber (5) provided with a wall (2) with a large number of holes with a cross section of 10-100 µm and communicating with a second chamber (6). The solution is pressed under the influence of a pressure differential between the first (5) and the second (6) chamber through the wall (2) with holes. The material for the cell sample is taken from the solution in the second chamber (6) for smearing on a slide. A suitable device for carrying out the process is also described.</p> 		

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Process and device for preparation of cell samples for
cytological tests

5 The present invention relates to a process for preparation
of cell samples for cytological tests of exfoliated cells,
and a device for carrying out the process.

10 Cytological tests of cell samples are now quite prevalent
and have proved to be an exceptional means for diagnosing
cell changes in the area about the portio-cervix and endo-
cervical canal. Such tests are performed on large numbers
of women and not infrequently on entire age groups of women
within a district for example.

15 There are also tests of cell samples taken from other
portions of the body done regularly at the cytological
laboratories, but no other test is as frequently done as
the cervical smear. A cell sample taken from the cervical
canal reveals cell changes with a reliability of about 95%,
20 and of these 20-25% are precancerous. A positive response
to the cell change test usually results in an operation,
a so-called scraping or use of a cone instrument. The
results of such treatment are good and well warrant extensive
testing of healthy women in certain age groups.

25 The samples are taken with a curette or similar instrument
by scraping cells from the mouth of the cervix. It is of
course very important that any cell changes be represented
in the sample. There are a number of different types of
30 sampling devices which provide acceptable samples. The
samples are taken by inserting a speculum into the vagina
whereafter the sampling instrument is inserted and the
scraping is done.

35 The sample material must then be protected from destruction
while being transported to the cytological laboratory,
suitably by immersion in a fixing solution.



After the sample has arrived at the laboratory it is prepared by dyeing and is inspected in a microscope. The presence of atypical cells is noted and reported. The examination under the microscope and the evaluation requires exceptional
5 attentiveness and is time-consuming, and is considered to be quite demanding work. In 1980 each test cost between 50 and 100 Swed.Kronor.

Consequently, intensive development work is in progress to
10 simplify and make less expensive sampling, sample preparation and sample evaluation. In order to simplify the evaluation of the samples under the microscope, a number of systems have been developed for automatic evaluation of cells as normal or atypical. Algorithms have been formulated for
15 automatic evaluation and the development of commercial systems is fairly far along.

It is thus possible with reasonably good accuracy to
20 determine if atypical cells are present in a cell sample, if the cell sample has been prepared so that a substantial portion of the cells are free cells on the slide. Under certain conditions, a human evaluator can also recognize atypical cells in clumps of cells, however.

This is not the case with automatic examination of a cell
25 sample and thus it is of crucial importance in this case to have as many free cells as possible. Samples have been prepared previously with a conventional syringe, for example, provided with a cannula with a diameter of 500 μ m, by
30 alternately sucking up and expelling the slurry of scraped cells. This produced cell samples which could be read by an experienced human examiner. In order to be read automatic-
ally, the sample preparation must be improved so that the majority of the cells in the sample are free cells.

35 The purpose of the present invention is thus to provide an effective process for sample preparation of cell samples and a device for carrying out the process.



The new process is essential for establishing a functioning system for automatic cell testing and facilitating substantially the non-automated evaluation of cell samples.

5 The new process, intended for the preparation of cell samples for cytological testing of exfoliated cells, in which the cell sample is in the form of a slurry in a fixing solution, is characterized in that the slurry is introduced into
10 a first chamber provided with a wall with a large number of holes with a cross section of 10-100 μm and in communication with a second chamber and that said solution, under the influence of a pressure differential, is made to pass through the wall provided with holes one or more times, and that
15 the material from the second chamber is used for the preparation of smears on a slide.

It is preferable that the cross section of the holes being 10-40 μm . If the cross section is less than 5 μm , the results
20 will not be reproducible, and if the cross section is more than 100 μm , the results of the test preparation will be less than satisfactory.

After one or more passages through the wall provided with holes, the solution is allowed to settle in the second
25 chamber and a smear on a slide is made from the sedimented material.

It has been shown to be suitable to use a pressure differential between the first and the second chambers of 50-1000
30 kPa to press the slurry through the wall provided with holes.

The pressure differential is suitably established by pressing a plunger into a cylindrical cavity, said cavity communicating with the first chamber.
35

The slurry suitably contains a cell dissociating agent, which can be for example hyaluronidase, chymotrypsin or



1,4-dimercapto-2,3-butane diol. Fixing agent, such as 50-95% ethanol is also included and suitably also a small amount of sodium hydroxide.

- 5 A device for carrying out the new process consists of a first and a second chamber separated by a wall, provided with a large number of holes with a cross section of 10-100 μm . It is also provided with means for establishing a pressure differential between the first and the second chamber.
- 10 Furthermore, it is arranged to make it possible to introduce slurry into the first chamber and to extract finished, possibly sedimented sample material from the second chamber. The device can consist of a first tube, closed at one end and into which a second tube has been partially inserted,
- 15 which at its inserted end is provided with a large number of holes and is essentially sealingly connected to the first tube. A movable plunger is arranged in the second tube and can be moved reciprocally there. Said plunger can also be inserted and removed from the second tube for introducing
- 20 the slurry into the second tube.

A preferred embodiment of the invention will be described in more detail with reference to the accompanying drawing.

- 25 The device consists of three parts: a tube 1 provided with a wall 2 with a plurality of holes, a plunger 3 and an outer closed tube 4. The tube 1 with the wall 2 and the plunger 3 defines the first chamber 5. The tube 1 can be inserted into the outer tube 4 suitably so as to seal against each other.
- 30 The second chamber 6 is defined in the tube 4 by the lower end of the closed tube and the wall tube provided with holes. The outer tube 4 is suitably a centrifuge tube with a pointed lower end 7. The tube 1 and the plunger 3 are suitably made as a hypodermic syringe with a flange 8 and a pressing
- 35 surface 9 as well as wings 10 for centering the movement of the plunger in the tube 1 when pressure is exerted on the pressing surface 9. The plunger 3 is suitably provided with



a rubber packing 11 to provide a better seal between the plunger 3 and the tube 1.

5 The device shown is intended for manual sample preparation, the plunger 3 first be removed from the tube 1 and the sample slurried in the physiological fluid being poured down into the tube 1. The plunger 3 is inserted and then pressed down through the tube 1. The solution will then be pressed through the wall with holes, suitably represented by a wire-mesh or
10 a net of artificial fibers with a cross section diameter of 10-100 μ m. The tubes 1 and 4 can then be turned over and the plunger 3 drawn out, thereby sucking the solution through the wall with holes, whereafter the liquid is again pressed with the plunger through the wall with holes. The process is
15 suitably repeated a number of times, whereafter the liquid is sedimented or centrifuged in the tube 4. The material for the smear can then be taken from the sedimental material thus providing a smear which has free cells to a large degree and which is suitable for automatic evaluation.

20

The person skilled in the art should have no difficulty conceiving automatic units in which the sample preparation can be done completely automatically even if we have not described here an example of such a device.

25



CLAIMS

1. Process for preparation of cell samples for cytological tests of exfoliated cells, in which the cell sample is in the form of a slurry in solution, characterized in that the slurry is introduced into a first chamber provided with a wall with a large number of holes with a cross section of 10-100 μm and in communication with a second chamber, and that said solution under the influence of a pressure differential between the first and the second chambers is made to pass through the wall provided with holes one or more times, and that the material from the second chamber is used for the preparation of smears on a slide.
2. Process according to claim 1, characterized in that the cross section of the holes is 10-40 μm .
3. Process according to claim 1, characterized in that a pressure differential of 50-1000 kPa is used to press the slurry through the wall provided with holes.
4. Process according to claim 1, characterized in that the liquid is allowed to sediment in the second chamber and that smears are made from the sedimental material.
5. Process according to claim 1, characterized in that the pressure differential is established by pressing a plunger into a cylindrical cavity, said cavity being in communication with the first chamber.
6. Process according to claim 1, characterized in that the slurry contains a cell dissociating agent and a fixing agent.
7. Process according to claim 6, characterized in that the cell dissociating agent consists of hyaluronidase, chymotrypsin or 1,4-mercapto-2,3-butane diol and the fixing agent of 50-95% ethanol and that the slurry contains a small amount of sodium hydroxide.



8. Process according to claim 1, characterized in that the slurry is made to pass through the wall by alternating pressure and suction effect.

- 5 9. Device for carrying out the process according to claim 1, characterized in that it consists of a first and a second chamber separated from each other by a wall provided with a large number of holes with a cross section of 10-100 μm ,
10 that it is provided with means for establishing a pressure differential between the first and the second chamber, that it is provided with an introduction opening for the slurry and means for removing prepared, possibly sedimented, sample material.
- 15 10. Device according to claim 9, characterized in that it consists of a first tube, closed at one end, into which a second tube has been partially inserted, said second tube being provided at its inserted end with a large number of holes and being substantially sealed against the first tube,
20 and a plunger which seals against the inner surface of the second tube and which can be inserted into and removed from the second tube and be moved back and forth therein.

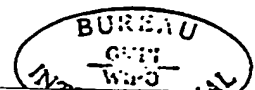


AMENDED CLAIMS
(received by the International Bureau on 22 December 1981 (22.12.81))

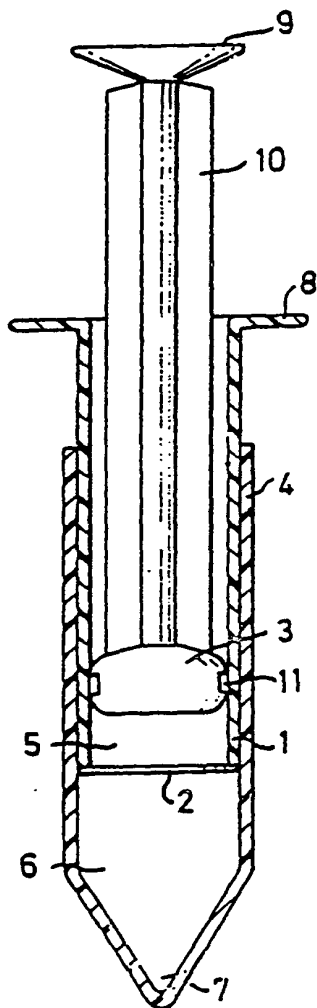
1. Process for preparation of cell samples for cytological tests of exfoliated cells, in which the cell sample is in the form of a slurry in solution, characterized in that the slurry is introduced into a first chamber provided with a wall with a large number of holes with a cross section of 10-100 μm and in communication with a second chamber, and that said solution under the influence of a pressure differential between the first and the second chambers is made to pass through the wall provided with holes one or more times, and that the material from the second chamber is used for the preparation of smears on a slide.
2. Process according to claim 1, characterized in that the cross section of the holes is 10-40 μm .
3. Process according to claim 1, characterized in that a pressure differential of 50-1000 kPa is used to press the slurry through the wall provided with holes.
4. Process according to claim 1, characterized in that the liquid is allowed to sediment in the second chamber and that smears are made from the sedimental material.
5. Process according to claim 1, characterized in that the pressure differential is established by pressing a plunger into a cylindrical cavity, said cavity being in communication with the first chamber.
6. Process according to claim 1, characterized in that the slurry contains a cell dissociating agent and a fixing agent.
7. Process according to claim 6, characterized in that the cell dissociating agent consists of hyaluronidase, chymotrypsin or 1,4-mercapto-2,3-butane diol and the fixing agent of 50-95% ethanol and that the slurry contains a small amount of sodium hydroxide.



8. Process according to claim 1, characterized in that the slurry is made to pass through the wall by alternating pressure and suction effect.
- 5 9. Device for carrying out the process according to claim 1, characterized in that it consists of a first and a second chamber separated from each other by a wall provided with a large number of holes with a cross section of 10-100 μm , that it is provided with means for establishing a pressure
10 differential between the first and the second chamber, that it is provided with an introduction opening for the slurry and means for removing prepared, possibly sedimented, sample material.
- 15 10. (Amended) Device according to claim 9, characterized in that it consists of a first tube, closed at one end, into which a second tube has been partially inserted, said second tube being provided at its inserted end with a large number of holes, and a plunger which seals against the inner
20 surface of the second tube and which can be inserted into and removed from the second tube and be moved back and forth therein.



1/1



INTERNATIONAL SEARCH REPORT

International Application No.

PCT/SE81/00199

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification and IPC

G 01 N 1/28

II. FIELDS SEARCHED

Minimum Documentation Searched

Classification System

Classification Symbols

IPC G 01 N 1/28-30, 31/00, 33/48, C 12 Q 1/01-02, A 61 B 10/00,
B 02 C 19/02-06, 12, 23/04-18,
US Cl 23:230 R, B, M, 253 R, 259; 128:1 R, 2 R .../...

Documentation Searched other than Minimum Documentation
to the extent that such Documents are included in the Fields Searched

SE, NO, DK, FI classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant Claim No. (s)
A	Patent Abstracts of Japan, abstract of JP 52-113793 published 1977, September 24, Hitachi Seisakusho K.K.	1-3
A	DE, A, 1 617 894 published 1971, April 8, Technicon Instruments Corp.	1-10
A	DE, A, 2 627 416 published 1977, January 20, Technicon Instruments Corp.	1-3
A	SE, B, 401 107 published 1978, April 24, L B Edebo	1-3
A	DE, B, 1 034 462 published 1958, July 17, G Klauke	1
A	US, A, 3 206 128 published 1965, September 14, Nordberg Manufacturing Company	1
X	US, A, 3 779 371 published 1973, December 18, Walter M Rovinski	9-10
X	DE, B, 1 034 886 published 1960, July 27, Roger Dreyfus	9-10

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"Y" document of critical importance the claimed invention cannot be considered to involve an inventive step when the document is compared with one or more other such documents, such combination being considered to a person skilled in the art

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IV. CERTIFICATION

Date of the Actual Completion of the International Search

Date of Mailing of this International Search Report

1981-10-22

1981-10-23

International Searching Authority

Signature of Authorized Officer

Swedish Patent Office

Solveig Arvidsson

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

II Continuation Fields Searched

US Cl 31:2, 26, 30, 69, 79, 83, 95.

III Continuation Documents considered to be Relevant.

X DE, A, 2 703 326 published 1977, October 6, 9-10
SEB S.A. Selongey

V. OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ... because they relate to subject matter not required to be searched by the Authority, namely:

2. ☐ Claim numbers ... because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all search subjects could be searched without effort justifying an additional fee, the International Searching Authority did not make payment of any additional fee.

Remarks on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.